

# Biochemical study of the population heterogeneity and distribution of the oval squid *Sepioteuthis lessoniana* complex in southwestern Japan

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**Abstract:** The three taxa of the *Sepioteuthis lessoniana* (Lesson, 1830) complex, AKAIKA, SHIROIKA, and KUAIKA, are genetically and reproductively independent and coexistent without hybridizing along the coast of Ishigaki Island, Okinawa. The present study analyzed 554 specimens of *Sepioteuthis* collected from 11 localities in inshore waters of southwestern Japan to elucidate the distributional patterns and population structures by means of horizontal starch gel electrophoresis at 13 genetic loci encoding for ten enzymes. The results showed that each of the three taxa has a different distributional pattern. SHIROIKA is widely distributed in the tropical to warm temperate regions throughout southwestern Japan. AKAIKA is distributed in the Ryukyu Islands and also probably occurs on the Pacific coast of Honshu, mainland Japan. KUAIKA is limited to the tropical region and its latitudinal distribution suggests a close correlation with water temperature. In SHIROIKA and KUAIKA, significant genetic differences were detected between the specimens from Ogasawara Islands and those from the other localities suggesting the existence of a certain barrier of panmixia between insular localities.

The oval squid, *Sepioteuthis lessoniana* (Lesson, 1830), is a loliginid squid widely distributed throughout the Indian Ocean and the western to central Pacific Ocean (Adam, 1939; Okutani, 1973). In Japan, this squid occurs in inshore waters extending from southern Hokkaido to Okinawa Islands (Sasaki, 1929; Okutani, 1973) and is one of the commercially important squids for neritic fisheries, especially in southwestern Japan (Dotsu *et al.*, 1981; Tsuchiya, 1982; Suzuki *et al.*, 1983; Ueta *et al.*, 1992).

On Ishigaki Island, Okinawa, southwestern Japan, fishermen have traditionally distinguished the oval squid into three different groups, namely, AKAIKA, SHIROIKA, and KUAIKA, based on the size, color in freshly-killed condition, and the fishing season and ground (Okutani, 1984; Segawa *et al.*, 1993a, b; Izuka *et al.*, 1994). Izuka *et al.* (1994) carried out allozyme electrophoresis resolving 11 loci in three groups of squids from Ishigaki Island. The results showed that the three groups differed from each other at least among three genetic loci. In addition, three types of egg capsules containing a different number of eggs per capsule were reported from Ishigaki Island (Segawa *et al.*, 1993a, b). Izuka *et al.* (1994) made it clear by allozyme electrophoresis that each group of squid produces different egg capsules: SHIROIKA produces egg capsules containing 4-8 eggs (mode = 6); KUAIKA lays two-egg capsules; and AKAIKA lays egg capsules containing 5-13 eggs (mean = 9.2; SD = 1.2). Further, each type of egg

capsule is laid on a different substratum (Segawa *et al.*, 1993a, b; Izuka *et al.*, 1994). These facts indicate that these three groups of *Sepioteuthis lessoniana* along the coast of Ishigaki Island are genetically and reproductively independent despite sympatry. Izuka *et al.* (1994) concluded that these three groups should be regarded as distinct species rather than as infraspecific fractions of a single species, *S. lessoniana*.

To date, allozyme electrophoresis has been a good tool for elucidating interspecific relationships (Augustyn and Grant, 1988; Brierley and Thorpe, 1994; Yokawa, 1994) and cryptic species (Smith *et al.*, 1981; Brierley *et al.*, 1993a; Yeatman and Benzie, 1993; Izuka *et al.*, 1994), and also providing useful information on population structure (*e.g.* Ally and Keck, 1978; Christofferson *et al.*, 1978; Garthwaite *et al.*, 1989; Brierley *et al.*, 1993b). The present study attempts to clarify the population structure and distribution of the three taxa currently referred to the *Sepioteuthis lessoniana* complex in southwestern Japan by allozyme analysis.

## MATERIALS AND METHODS

A total of 554 specimens of *Sepioteuthis* were collected from ten localities in inshore waters of southwestern Japan and a single site at Rayong in the Gulf of Thailand by

**Table 1.** Sampling data for squids used in the present study. Abbreviation of locality as mentioned in tables and figures are in parentheses.

Sampling Locality	Abbreviated Locality Name	N	Date of Collection
Ishigaki Island, Okinawa Pref.	(Ishigaki)	83	May-Oct. 1992
Amami Island, Kagoshima Pref.	(Amami)	22	Feb.-Mar. 1994
Ogasawara Islands, Tokyo	(Ogasawara)	178	June-Oct. 1994
Miyazu City, Kyoto	(Kyoto)	39	Oct. 1992
Sakai, Toyama Pref.	(Toyama)	17	Oct. 1992
Tsuruga, Fukui Pref.	(Fukui)	20	Oct. 1993
Nagato, Yamaguchi Pref.	(Yamaguchi)	36	Feb. 1993
Miura Peninsula, Sagami Bay	(Sagami)	8	July 1994
Hiwasa, Tokushima Pref.	(Tokushima)	102	Aug.-Dec. 1994
Shima, Mie Pref.	(Mie)	45	Aug. 1994
Rayong, Gulf of Thailand	(Thailand)	4	Jan. 1994
	Total	554	

set net, jigging, or scoop net during the period from May 1992 to December 1994 (Fig. 1, Table 1). The squid samples from Ishigaki Island used in the present study were the same as those studied by Izuka *et al.* (1994) with additional genetic data for three loci of *AAT-2\**, *G3PDH\**, and *PGM\**. All the samples were transferred immediately after collection to the laboratory of Tokyo University of Fisheries and kept frozen at  $-80^{\circ}\text{C}$  until analysis. Horizontal starch gel electrophoresis was carried out using buccal mass muscle (for details of methods, see Izuka *et al.*, 1994). Three

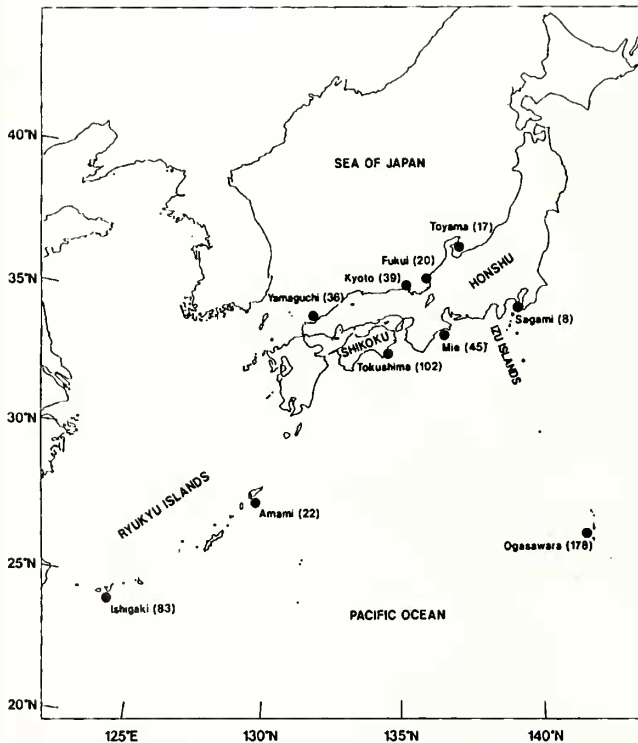
buffer systems described by Numachi (1989) were used for electrode and gel: citrate-N-(3-aminopropyl) morpholine buffer at pH 6 and pH 7 (CAPM6 and CAPM7) and tris-citric acid buffer at pH 8 (CT-8N).

After electrophoresis, the gel was stained for ten enzymes (Table 2). Staining protocols were cited from Hillis and Moritz (1990) except for diaphorase which followed Harris and Hopkinson (1976). Nomenclature of locus and allele follows the guidelines of Shaklee *et al.* (1990). All the samples were classified into AKAIKA, SHIROIKA, and KUAIKA on the basis of asparate aminotransferase genotypes which are taxon-specific (Izuka *et al.*, 1994).

Differences in allele frequencies among localities within every combination of taxa were tested for significance ( $P < 0.05$ ) using the chi square test (Kimura, 1960). Intrapopulational variability was evaluated by the proportion of polymorphic loci (P) (5% level) and heterozygosity (H). However, heterozygosity was not analyzed when the sample size was  $< 15$  specimens for statistical reasons (Nei, 1987). Nei's (1972) genetic distance (D) was calculated from the data of allele frequencies, and the unweighted paired group method of cluster analysis (Sokal and Sneath, 1963) was employed to establish genetic relationships among the squids sampled.

## RESULTS

We detected 47 individuals of AKAIKA from three localities, 470 SHIROIKA from 11 localities, and 37 KUAIKA from three localities (Fig. 2). No other kinds of oval squid were found in the present materials. The three taxa could be completely identified by fixed genetic differences at *AAT-1\** (Table 3). Taxon-specific alleles were also recognized at *DIA\** and *IDHP\** in SHIROIKA, and at



**Fig. 1.** Sampling localities of the specimens examined in the present study. Numbers in parentheses indicate number of specimens analyzed.

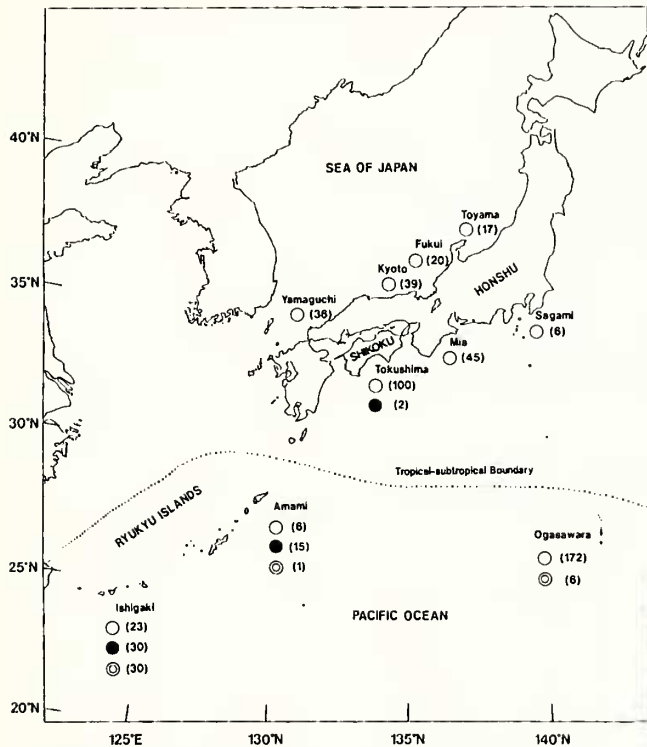


Fig. 2. Distribution of each of the three taxa as detected by electrophoresis. ○, SHIROIKA; ●, AKAIKA; ⊙, KUAIKA. Numbers in parentheses indicate number of specimens detected.

*MDH-1\** and *SORD\** in KUAIKA (Table 3).

### AKAIKA

AKAIKA squid were found in samples from Tokushima, Shikoku, and Amami and Ishigaki Islands (Fig. 2). No significant difference in allele frequency was detected among these squids, and genetic distances among these sites were close to zero (Fig. 3). All loci were monomor-

phic except for a single *PGDH\** heterozygote from Ishigaki Island (Table 3). These observations indicate that the squids of these three localities are not differentiated from each other and may share a common gene pool.

### KUAIKA

KUAIKA was detected from the Ryukyu and Ogasawara Islands, and never observed in the seven samples from inshore waters around Honshu (Fig. 2). No significant genetic difference was detected between the specimens from Amami and Ishigaki Islands indicating that the KUAIKA squid population within the Ryukyu Islands is genetically uniform. However, a significant difference in allele frequency was detected by chi square test at *PGM\** between the squids of Ryukyu Islands and Ogasawara Islands. The Ogasawara Islands specimens were fixed for *PGM\*a* while only a few heterozygotes from Ishigaki Island exhibited this allele (Table 3). Genetic distance between the populations of Ogasawara Islands and those of Ryukyu Islands was 0.0689 (Fig. 3). Genetic variation was observed only in the squids of Ishigaki Island (Table 3) but the other samples are too small for comparison.

### SHIROIKA

SHIROIKA squid were widely distributed through inshore waters around Honshu and from the Ogasawara and Ryukyu Islands, and in the Gulf of Thailand (Fig. 2). Except for the Ogasawara Islands sample, no significant genetic differences were observed among these populations and genetic distances were close to zero (Fig. 3). These facts suggest that SHIROIKA squid from Honshu, Shikoku, Ryukyu Islands, and Thailand could share common gene pool over their 2000 km geographical range. In contrast, slight but significant differences of allele frequencies were detected between the squids of Ogasawara Islands and those

Table 2. List of enzymes, loci examined, and buffers used for electrophoresis.

Enzyme	E.C. No.	Abbreviation	Loci	Buffer
Aspartate aminotransferase	2.6.1.1	AAT	<i>AAT-1*</i>	CAPM6
			<i>AAT-2*</i>	"
Diaphorase	1.6	DIA	<i>DIA*</i>	"
Glycerol-3-phosphate dehydrogenase	1.1.1.8	G3PDH	<i>G3PDH*</i>	CAPM7
Glucose-6-phosphate isomerase	5.3.1.9	GPI	<i>GPI*</i>	CAPM6
Isocitrate dehydrogenase	1.1.1.42	IDH	<i>IDH*</i>	CAPM7
Malate dehydrogenase	1.1.1.37	MDH	<i>MDH-1*</i>	"
			<i>MDH-2*</i>	"
			<i>MDH-3*</i>	"
Mannose-6-phosphate isomerase	5.3.1.8	MPI	<i>MPI*</i>	CT-8N
6-Phosphogluconate dehydrogenase	1.1.1.44	PGDH	<i>PGDH*</i>	CAPM7
Phosphoglucomutase	5.4.2.2	PGM	<i>PGM*</i>	CAPM6
Sorbitol dehydrogenase	1.1.1.14	SORD	<i>SORD*</i>	CAPM7

Table 3. Allele frequency, proportion of loci polymorphic (P), and heterozygosity (H) at 13 genetic loci by locality for three groups.

Locus	Allele	AKAIKA					KUIAIKA					SHIROIKA						
		Ishigaki	Amami	Tokushima	Ishigaki	Amami	Ogasawara	Ishigaki	Amami	Ogasawara	Kyoto	Toyama	Fukui	Yamaguchi	Sugmi	Tokushima	Mie	Thailand
AAT-1*	*a	-	-	-	-	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	*b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AAT-2*	*c	1.00	1.00	1.00	1.00	1.00	(6)	(6)	(171)	(39)	(17)	(18)	(31)	(8)	(100)	(45)	(4)	
	*a	(30)	(15)	(2)	(30)	(1)	(6)	(22)	(6)	(171)	(39)	(17)	(18)	(31)	(8)	(100)	(45)	(4)
DIA*	*a	1.00	1.00	1.00	1.00	1.00	(4)	(4)	(79)	(38)	(4)	(1)	(2)	(8)	(93)	(45)	(4)	
	*b	(7)	(13)	(2)	(22)	(1)	(3)	(4)	(79)	(38)	(4)	(1)	(2)	(8)	(93)	(45)	(4)	
G3PDH*	*a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
	*b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
GPI*	*a	(30)	(15)	(2)	(30)	(1)	(6)	(23)	(172)	(39)	(17)	(19)	(22)	(8)	(100)	(45)	(4)	
	*b	(3)	(15)	(2)	(8)	(1)	(6)	(4)	(172)	(38)	(17)	(17)	(28)	(8)	(100)	(45)	(4)	
IDHP*	*a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
	*b	(15)	(15)	(2)	(30)	(1)	(6)	(17)	(171)	(37)	(6)	(18)	(21)	(8)	(100)	(45)	(4)	
MDH-1*	*a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
	*b	(30)	(15)	(2)	(30)	(1)	(6)	(23)	(171)	(39)	(17)	(20)	(36)	(8)	(100)	(45)	(4)	
MDH-2*	*a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
	*b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
MDH-3*	*a	(30)	(15)	(2)	(30)	(1)	(6)	(18)	(171)	(39)	(17)	(20)	(36)	(8)	(100)	(45)	(4)	
	*b	(30)	(15)	(2)	(30)	(1)	(6)	(18)	(171)	(39)	(17)	(20)	(36)	(8)	(100)	(45)	(4)	
MPI*	*a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
	*b	(25)	(15)	(2)	(30)	(1)	(6)	(12)	(121)	(39)	(17)	(20)	(35)	(8)	(100)	(45)	(4)	
PGDH*	*a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
	*b	(30)	(15)	(2)	(30)	(1)	(4)	(23)	(163)	(39)	(17)	(19)	(36)	(8)	(100)	(45)	(4)	
PGM*	*c	0.02	0.02	-	-	-	0.06	0.06	0.53	0.14	0.12	0.13	0.12	0.12	0.05	0.07	-	
	*d	0.98	1.00	1.00	1.00	1.00	0.93	0.93	0.47	0.82	0.79	0.83	0.86	0.94	0.89	0.82	1.00	
SORD*	*a	-	-	-	-	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
	*b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
P	*c	(30)	(15)	(2)	(30)	(1)	(6)	(23)	(171)	(39)	(17)	(20)	(3)	(8)	(100)	(45)	(4)	
	*d	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	0.90	0.97	1.00	0.94	0.94	1.00	
H	*a	0.15	0.15	0.085	0.15	0.15	0.08	0.08	0.08	0.01	0.03	0.10	0.03	0.06	0.05	0.04	-	
	*b	0.85	0.85	0.915	0.85	0.85	0.92	0.92	0.92	0.99	0.97	0.90	0.97	0.94	0.95	0.94	1.00	
P	*c	(24)	(15)	(2)	(20)	(1)	(6)	(21)	(172)	(38)	(17)	(19)	(32)	(8)	(100)	(45)	(4)	
	*d	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.91	0.87	0.94	0.95	1.00	0.92	0.92	1.00	
H	*a	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	
	*b	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	
P	*c	(22)	(15)	(2)	(30)	(1)	(6)	(16)	(172)	(39)	(16)	(18)	(29)	(8)	(100)	(45)	(4)	
	*d	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.91	0.87	0.94	0.95	1.00	0.92	0.92	1.00	
H	*a	0.00	0.00	0.00	0.00	0.00	0.08	0.08	0.08	0.15	0.15	0.23	0.15	0.02	0.23	0.23	-	
	*b	0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.02	0.02	0.05	0.04	0.02	0.02	0.03	0.04	-	

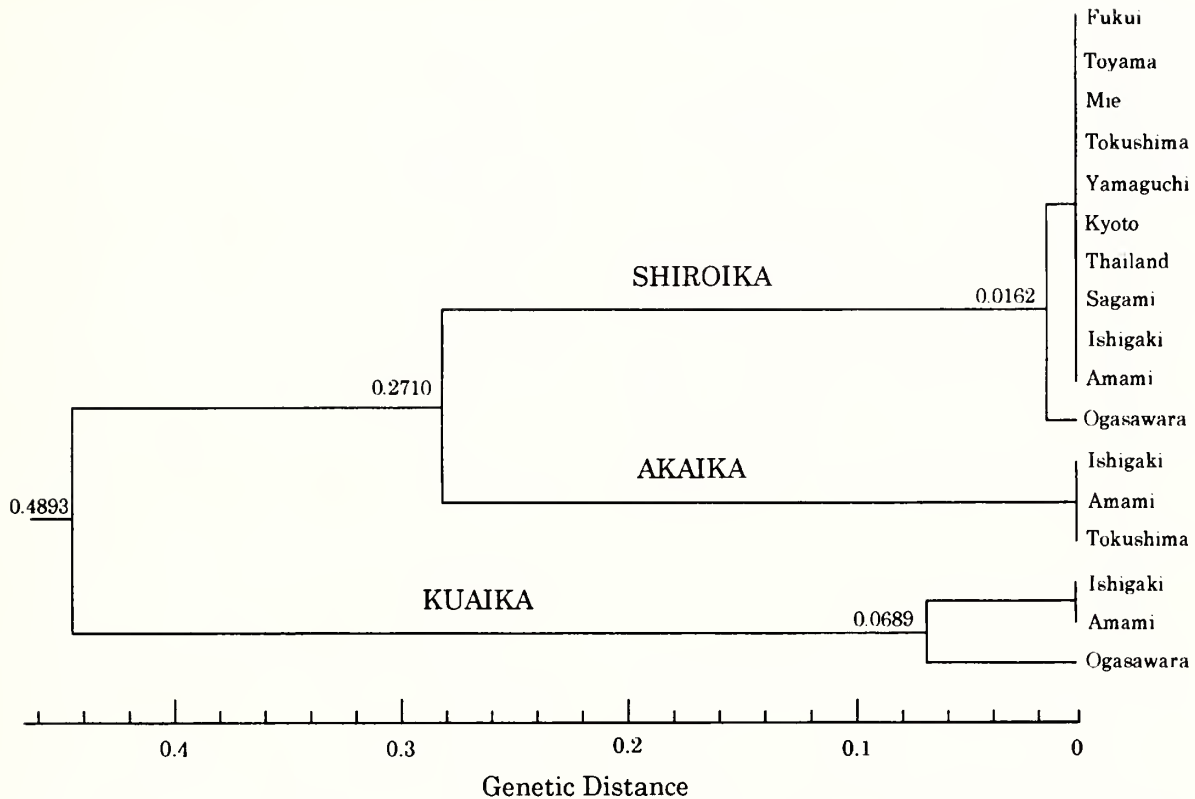


Fig. 3. Biochemical similarity dendrogram based on genetic distance among the squids by locality (for details on localities, see Table 1 and Fig. 1).

of the other localities ( $D = 0.0162$ ) (Fig. 3).

Genetic variation was detected in all Japanese samples large enough to be tested. Mean heterozygosity ( $H$ ) of the squids around Honshu was 0.037 (0.023-0.046) and the proportions of polymorphic loci ( $P$ ) was 0.193 (0.154-0.231) (Table 3). Samples from Ishigaki Island and the Ogasawara Islands were slightly less variable (Table 3).

## DISCUSSION

The present study clarified the distributional pattern of each taxon in the *Sepioteuthis lessoniana* complex around southwestern Japan. SHIROIKA is the common widely distributed squid in the tropical to warm temperate region of northwestern Pacific. AKAIKA was detected from Ryukyu Islands and the Pacific coast of Shikoku but probably extends easterly to the Izu Islands as egg capsules containing 6-12 eggs have been observed on deeper bottoms (40-50 m) there (Izuka, unpubl.). KUAIKA was the only taxon restricted to the Ryukyu and Ogasawara Islands and was never found around the main Japanese islands. Amami and Ogasawara Islands are both located in the

northernmost part of the tropical region (Briggs, 1974; Nishimura, 1992) and lie near the isotherm of minimum winter temperature of about 20°C (*e. g.* Briggs, 1974; Dall, 1991). Izuka *et al.* (1994) assumed that KUAIKA were confined to the tropical western Pacific, because their egg capsules have been observed only on shallow coral reefs in Ishigaki Island, Okinawa Island, Palau Island, and New Guinea. These facts suggest that KUAIKA is a tropical squid which extends north to the tropical-subtropical boundary (Fig. 3).

Genetic differences appeared in allele frequencies of both SHIROIKA and KUAIKA between Ogasawara Islands and the other sampling localities. This fact may be evidence of a certain barrier against panmixia between these localities. Brierley *et al.* (1993b) found that populations of *Loligo forbesi* Steenstrup, 1856, from the British Isles and the Azores could be considered to be existing in allopatry because of large distance, oceanic depths, and ocean currents between sites. It is unlikely that SHIROIKA and KUAIKA in the Ogasawara Islands maintain sufficient gene flow with those of the mainland and Ryukyu Islands, as the populations of these two areas are separated (more than 1000 km) by the Kuroshio Current. If dispersal of

KUAIKA had taken place between these areas, allele \*b which was recognized as the common allele at *PGM\** in the Ryukyu Islands should be detected in the Ogasawara Islands population (Table 3). It suggested that KUAIKA in the Ogasawara Islands is almost genetically segregated from the Ryukyu Islands.

The present study revealed that each of the three taxa of the *Sepioteuthis lessoniana* complex has a different distributional pattern. Within two of these taxa, the populations from the Ogasawara Islands are genetically a little different from those of the other areas sampled. However, the sampling sites in the present study were restricted on the northernmost rim of the western central Pacific. More work including investigations at more southern localities is now required to estimate interpopulational variabilities over the entire geographical range of each of the three taxa.

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